Deconstruction of septin assembly

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Cells constantly undergo shape changes that are necessary to perform their functions, such as cell migration, cell division, and tissue (re)generation. Cytoskeletal proteins play a crucial role in driving these shape changes, yet at the same time they maintain the mechanical integrity of the cell. Microtubules, intermediate filaments and actin filaments are the main components of the cytoskeletal machinery of animal cells. These proteins are all filamentous, but differ in their spatial distribution in the cell, and in their dynamical and mechanical properties. The main cytoskeletal component that drives cell shape changes is actin, which forms a thin crosslinked meshwork at the cell cortex, right underneath the plasma membrane. Myosin motor proteins exert forces on actin filaments and drive actin network contraction. However, for contraction to result in cell shape changes, the actomyosin cortex has to be linked to the plasma membrane.

In this thesis we focus on an understudied cytoskeletal protein known as septin, which likely acts as a linker between the actomyosin cortex and the plasma membrane. Like actin, septin is found mostly at the cell cortex, forms filamentous structures, and is important to maintain cell rigidity. Septins are important in a variety of cell functions that involve cell shape changes, like cell division and migration. They interact with actin and microtubules, contributing to cytoskeletal crosstalk. Moreover, septins provide diffusion barriers at specialized regions of the cell membrane, especially in regions where the plasma membrane exhibits high curvature. The available data suggest that 1) septins themselves can locally influence cell shape and rigidity, and that 2) septins can act as linkers of actomyosin to the plasma membrane, allowing for the transduction of actomyosin forces directly to the plasma membrane. However, there is a lack of knowledge on the assembly properties of septins, alone and in the presence of lipid membranes.

The main goal of this thesis is to understand how septins assemble into higher order filamentous structures, and how these structures allow septins to perform their functions at the cell cortex. To simplify the complex cell environment, we design *in vitro* reconstitution experiments based on recombinantly expressed septins from the genetic model organism *Drosophila melanogaster*.

In **Chapter 2** we characterize septin self-assembly in bulk solution. We use fluorescence microscopy to investigate fly septin bundle formation across different concentrations and compare this with the so far better characterized assembly properties of yeast septins. We find that fly septins form rigid bundles above an onset concentration of 200 nM, while yeast septins form paired filaments at concentrations that are 10 times lower. We investigate further features of the septin bundles using STEM, an electron microscopy method that permits spatially resolved measurements of the protein mass. First, we confirm prior findings that yeast septins form predominantly paired filaments in solution. Then we characterize fly septin bundles formed at high septin concentrations, and find that they form thick structures up to 250 nm in width and with tapered ends. By mass mapping, we calculate that these bundles are composed of hundreds of hexamers per cross-section. Close to the onset concentration for bundling (200 nM), we observe similar tapered bundle structures, although

thinner (∼20 hexamers per cross-section), and we also find another type of bundle, with a constant width of ∼60 nm, blunt ends, around 6 to 8 subunits per cross-section, and a characteristic groove along the long axis. These findings allow us to speculate about the intermediate steps of septin bundle assembly.

In **Chapter 3** we characterize septin assembly in the presence of biomimetic lipid membranes. We observe that in contrast to the thick bundles formed in solution, septins form a dense layer of thin filaments on lipid bilayers containing negatively charged lipids. We show that the septin-lipid interaction is electrostatic in origin, unlike in the case of yeast septins, which specifically bind phosphoinositide lipids. To resolve the ultrastructural details of the membrane-bound septin layers, we turned to higher resolution microscopy techniques. Transmission electron microscopy (negative stain and cryoEM) revealed that septins form single and paired filaments, which are tightly packed together in flat, ribbon-like bundles. Atomic force microscopy (AFM) imaging in liquid showed that the septin film thickness increased from 4 nm at 12 nM septins to 18 nm for 60 nM septins. Furthermore, we showed that flat ribbon-like septin bundles are resistant to the mechanical force exerted by the AFM tip while scanning, whereas single filaments are more prone to get dislocated. This finding suggests that lateral interactions between septin filaments impart mechanical strength to septin films. Our findings prove that membrane binding catalyzes septin higher order assembly and that septin-lipid interactions compete with septin-septin interactions in the solution. These findings provide new evidence that the presence of septins at the cell cortex may be (at least in part) driven by direct interactions with the plasma membrane and that membrane-bound septins may potentially contribute to cortical rigidity.

In **Chapter 4** we characterize the kinetics of membrane-binding of septins using a surface analytical technique known as quartz-crystal microbalance with dissipation monitoring (QCM-D). We show that septin binding to the lipid bilayer is driven by electrostatic interactions and is mass transport limited. Furthermore we show that septins do not desorb upon washing with buffer, suggesting a strong interaction with the lipid bilayer. We also show that the septin layer is thin (being composed of at most 3 septin layers), relatively rigid, and self-limited in growth. We directly confirm by AFM nanoindentation measurements that the septin films have a maximum thickness of 14 nm. By combining QCM-D with concurrent optical measurements of septin adsorption by spectroscopic ellipsometry, we reveal that the septin films contain a large (80%) mass fraction of hydrodynamically coupled solvent, consistent with the porous architecture of the filamentous films. All these findings together allow us to propose a model of septin higher order assembly on model lipid membranes, which may guide future studies of the architecture and mechanical functions of septins at the cell cortex.